ELSEVIER

International Journal of Pharmaceutics 132 (1996) 143–151

international journal of pharmaceutics

An FTIR study of the structure of human serum albumin adsorbed to polysulfone

Paul M. Bummer*

Division of Medicinal Chemistry and Pharmaceutics, College of Pharmacy, and Center for Membrane Sciences, University of Kentucky, Lexington, KY 40536-0082, USA

Received 11 August 1995; revised 16 October 1995; accepted 17 October 1995

Abstract

Internal reflection infrared spectroscopy was employed in a study of the structural changes in human serum albumin that result from adsorption to cast films of polysulfone. Adsorption experiments were carried out in a flow-through cell at 30°C in the presence of either a H_2O-based or D_2O-based saline buffer. Spectral features show adsorption results in Amide I bandwidth broadening and diminished Amide II band intensities relative to the Amide I band. Deconvolution and band-fitting of spectra obtained in the presence of D_20 show appearance of beta and aperiodic conformations at the expense of helical structures. The results suggest considerable change in the secondary structure of human serum albumin upon adsorption to polysulfone.

Keywords: Protein adsorption; Protein conformation; Human serum albumin; Polysulfone

1. Introduction

Protein adsorption to membrane filters and the subsequent fouling of the device continues to be a major obstacle to the application of membrane technologies (Bowen and Gan, 1992; Brites and dePinko, 1993; Meirles et al., 1991; Campbell et al., 1993; Palecek and Zydney, 1994). Adsorption to membrane surfaces has been shown to influence adversely the stability and biological activity of protein and peptide pharmaceuticals (Bowen and Gan, 1992; van den Oetelaar et al., 1989; Pitt, 1987; Devis et al., 1990). In the food industry, filter fouling by surface-adsorbed proteins results in increased processing costs (Nilsson, 1990; Nystrom, 1989). In the field of biomaterials, adsorption-associated structural changes of blood proteins are thought to be one of the important events initiating thrombus formation on polymeric surfaces of implants and membrane-based blood oxygenators (Baier and Dutton, 1969; Ihlenfeld and Cooper, 1979).

Membrane filters composed of polysulfone (PSf) appear to be especially vulnerable to protein fouling (Brites and dePinko, 1993; Robertson and Zydney, 1990; Oldani and Schock, 1989; Hanemaaijer et al., 1989; Wahlgren and Arnebrant, 1990). The molecular structure of polysulfone is shown in Fig. 1. Several groups have studied the

* Tel. +1 606 257 8881; Fax +1 606 257 7585; E-mail pbumm01@pop.uky.edu.

SSDI 0378-5173(95)04344-A

interactions of proteins with commercially-available polysulfone-based ultrafiltration membranes. Such membranes are asymmetric, composed of an ultrathin skin, a substructure and a membrane matrix, often of different materials (Robertson and Zydney, 1990). The complex nature of the membrane complicates greatly the analysis of adsorption (Nilsson, 1990; Robertson and Zydney, 1990; Oldani and Schock, 1989; Hanemaaijer et al., 1989; Wahlgren and Arnebrant, 1990). Even in those instances where the membrane is fabricated from a single polymer, the influences of membrane pores are difficult to assess (Robertson and Zydney, 1990; Hanemaaijer et al., 1989). This is especially important in that a complex interplay of surface adsorption and shear in the pore may influence the fouling characteristics of the membrane (Meirles et al., 1991; Campbell et al., 1993). Considerable efforts have been extended to the formidable task of full characterization of pristine and fouled membrane products by surface spectroscopic techniques (Oldani and Schock, 1989; Keurentjes et al., 1989; Fontyn et al., 1987, 1991). Complimentary studies of protein adsorption to dense, pore-free films of polysulfone have not been carried out, but offer the advantage of a less complex morphology and composition.

The native three-dimensional structure of a protein in solution is the result of a complex balance of repulsive and attractive forces (Creighton, 1984). Surfaces are believed to disrupt the balance of forces in a protein residing in an interfacial region, resulting in the structure of the adsorbed molecule being altered compared to the native state (Andrade, 1985). The strong attachments that proteins tend to show for surfaces are thought to be due, in part, to structural changes that result in a greater number of attachment points (Andrade, 1985). Although proteins are known to adsorb very rapidly to polymeric surfaces, the associated structural changes are observed on a much longer timescale (Andrade, 1985; Chittur et al., 1986; Lenk et al., 1989). The objective of the present study is to examine, by an infrared spectroscopic approach, the adsorption of human serum albumin (HSA) to a cast film of polysulfone (Chittur et al., 1986; Lenk et al., 1989). Infrared spectroscopy has been shown to provide a convenient and sensitive means of probing structural features of proteins both in solution and adsorbed to polymeric surfaces (Lenk et al., 1989; Surewicz and Mantsch, 1988). Such studies should provide valuable insight into the molecular details of protein behavior in ultrafiltration membranes.

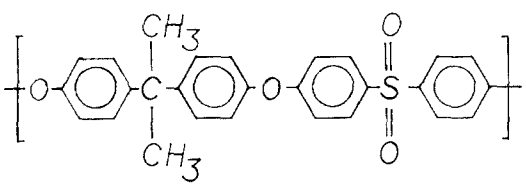

Fig. 1. Molecular structure of polysulfone.

2. Experimental

2.1. Film casting

A thin dense film of polysulfone (Udel 1700, Union Carbine, Inc.) was coated onto zinc selenide rods (3 mm diameter, 25 mm length) by a dipping procedure (Pitt and Cooper, 1988). The polymer solution concentration was 0.5% (w/v) in chloroform and the withdrawal rate was 0.5 mm/min. The relative humidity of the dipping chamber was maintained at less than 8% by a dry air purge. The coating was dried for 1 h at 60°C and returned to room temperature prior to use. Due to the cylindrical shape of the ATR crystal, it was not possible to employ spectroscopic methods to assess reproducibility of the coating. As an assessment of reproducibility of coating, the mass of polymer deposited was determined by subtracting the mass of the clean crystal from the mass of the polymer-coated crystal. From the mass density of the polymer and the geometry of the crystal, an average film thickness was calculated to be 1.2 ± 0.4 microns. No film thickness was every found to be less than 0.6 microns.

2.2. Materials

Human serum albumin (HSA, crystallized and lyophilized, Sigma Chemical) was used as re-

ceived. For adsorption studies, solutions of the proteins were made in 0.9% NaCl/1 mM phosphate buffer (pH 7.0) in water (H_2O buffer) or deuterium oxide (D_2O buffer), to a final concentration of 1 mg/ml. The solution spectra of HSA were determined in the same buffers at a final protein concentration of 10 mg/ml.

2.3. Spectral analysis

All spectra were collected on a Digilab FTS-40 Fourier-transform infrared spectrometer (1024 scans at 4 cm^{-1} resolution, 30 ± 1°C). The coated rod was mounted in a stainless steel MicroCircle cell (Spectra-Tech, Stamford, CT) and a background spectrum was obtained of the crystal and polymer. Buffer was pumped through the cell (syringe pump, Sage Instruments) at a rate of 3 ml/h and a background spectrum obtained. The wall shear rate was estimated to be approximately 100 s^{-1} (Bird et al., 1960). Protein solution was then pumped through the cell at the same flow rate for 4 h. Preliminary experiments showed that most spectral changes of adsorbed protein were completed after 4 h of exposure. Buffer and protein solutions were not recirculated. To remove loosely-bound protein, the solution was replaced with flowing H_2O buffer for 20 min, after which a spectrum of adsorbed protein + buffer was obtained. The H_2O-based buffer was then replaced with D_2O buffer (pD adjusted to 7.0 with DCl) and a spectrum of deuterated protein + D_2O-based buffer immediately collected. Each PSf film was exposed to HSA only once and the experiments were repeated a total of five times. The spectrum of the tightly-bound protein was obtained by digital subtraction of the appropriate buffer spectrum from that of the adsorbed protein + buffer (Powell et al., 1986). Spectral manipulations were carried out employing Digilab software. Overlapping infrared bands were resolved by Fourier self-deconvolution techniques employing a Lorentzian lineshape of 25 cm^{-1} full width at half-height and a resolution enhancement factor of 2 (Mantsch et al., 1986). Band-fitting analysis was carried out also employing DigiLab software. Input parameters were the component band positions determined by the deconvolution analysis. Band positions were not permitted to vary during the analysis. Component band widths and intensities were varied by the software to minimize the root mean square difference between the experimental spectrum and the summation spectrum of the component bands.

3. Results and discussion

In the mid-infrared range, the spectra of polypeptides are comprised of three regions, the Amide I (1700–1600 cm^{-1}), the Amide II (1600–1500 cm^{-1}) and the Amide III (1320–1230 cm^{-1}) (Parker, 1983). The Amide II band absorbance intensity has been reported to be proportional to the amount of protein adsorbed (Surewicz and Mantsch, 1988), and is believed not to be very sensitive to the conformation of the protein (Parker, 1983). Since the Amide III band is approximately an order of magnitude less intense that the Amide I band, it will not be considered further. In the Amide I region, the observed stretching frequency of C = O hydrogen-bonded to NH moieties is dependent upon the secondary structure adopted by the peptide chain (Surewicz and Mantsch, 1988; Mantsch et al., 1986). Changes in the structure of the protein are reflected by changes in the component band positions of the Amide I region. Based on the work of a number of authors (Parker, 1983; Susi and Byler, 1983; Byler and Susi, 1986; Jackson et al., 1989; Krimm and Bandekar, 1986), component bands in the region of 1650 to 1658 cm^{-1} (in water) are assigned to alpha-helices. The infrared spectra of beta structures usually exhibit a doublet with a low intensity component in the region of 1670 to 1695 cm^{-1} and a higher intensity component in the region of 1620 to 1640 cm^{-1}. In H_2O, aperiodic conformations exhibit absorbance bands in the 1650 to 1660 cm^{-1} region. Overlapping of the helix and aperiodic regions often makes structural assignments difficult (Susi and Byler, 1983; Byler and Susi, 1986).

Presented in Fig. 2 are the Amide I and the Amide II regions of a typical infrared spectrum of HSA in the solution state and adsorbed to PSf (H_2O buffer). Several differences are immediately

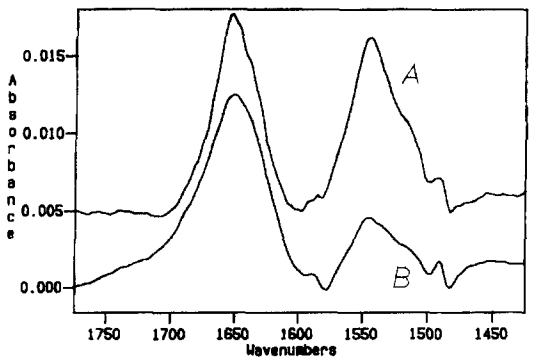

Fig. 2. Infrared spectrum of the Amide I and Amide II regions of human serum albumin in H_2O-based buffer solution. (A) Spectrum of the protein in solution; (B) Spectrum of the protein adsorbed to cast polysulfone film.

evident when the two spectra are compared. In the solution state, the relative absorbance intensities of the Amide I and Amide II bands centered at 1652 and 1545 cm^{-1} respectively, are approximately equal. In the case of the adsorbed protein, the intensity of the Amide I band (1651 cm^{-1}) is more than twice that of the Amide II band (1547 cm^{-1}). Differences in the intensity ratio of Amide I to Amide II peaks between the solution and adsorbed proteins have been attributed to unspecified differences in secondary and tertiary structures (Lenk et al., 1989; Wasacz et al., 1987; Ishida and Griffiths, 1993). The Amide I bandwidth at 70% of band height is greater for the adsorbed HSA versus that for the protein in the solution state (42 ± 1 and 27 ± 2 cm^{-1}, respectively). Band broadening upon adsorption indicates a wider range of vibrational frequencies, implying some sort of structural change relative to the solution state (Lenk et al., 1989). The spectral alterations noted in the present study do not overlap the major infrared peaks for buffer salts, indicating that the observed changes are not an artifact of concentration or dilution of buffer salts in the evanescent wave (Lenk et al., 1989). The increased intensity of Amide I relative to Amide II and the Amide I bandwidth broadening exhibited by adsorbed HSA are similar to those features observed for other proteins on other polymeric surfaces (Pitt and Cooper, 1988; Powell et al., 1986; Mantsch et al., 1986).

To compare more meaningfully the structure of the protein in solution and in the adsorbed state, band splitting of the Amide I region was examined by Fourier self-deconvolution. In the solution state (Fig. 3A), spectral deconvolution reveals component bands at 1682, 1654 and 1635 cm^{-1} (Table 1). These bands may be tentatively assigned to beta structures (1682 and 1635 cm^{-1}) and to helix/aperiodic structures (1654 cm^{-1}) (Parker, 1983; Susi and Byler, 1983; Byler and Susi, 1986; Jackson et al., 1989). Part of the band at 1635 cm^{-1} may also represent short extended chains connecting helical segments (Surewicz and Mantsch, 1988). This is in qualitative agreement with the solution structure of HSA which is approximately 55% helix, 35% aperiodic and 10% beta structure (Carter and Ho, 1994). For the

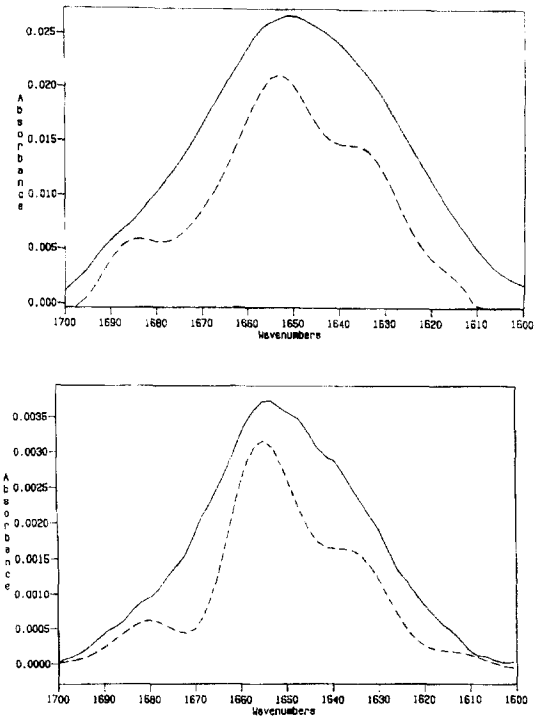

Fig. 3. Infrared spectrum of the Amide I region of human serum albumin in H_2O-based buffer. (A) Full line, spectrum of native protein in solution; dashed line, spectrum of native protein in solution after Fourier self-deconvolution, bandwidth 25 cm^{-1}, enhancement factor 2. (B) Full line, spectrum of protein adsorbed to cast dense polysulfone film; dashed line, spectrum of adsorbed protein after Fourier self-deconvolution.

Table 1
Characteristic Amide I component bands in the infrared spectrum of HSA in solution and adsorbed to PSf in the presence of H_2O buffer

	Observed wavenumber[a] (cm^{-1})	Proportion[b] (%)	Tentative assignment
Solution	1682	7	Beta structure
	1654	68	Helix/aperiodic
	1635	24	Beta structure
	1616	1	
Adsorbed	1740[c]		Carboxyl group
	1708[c]		Carboxyl group
	1687	5	Beta structure
	1670 (shoulder)[c]		
	1654	45	Helix/aperiodic
	1634	40	Beta structure
	1615	9	

[a]After deconvolution, see text for details.
[b]Average percent areas obtained by curve fitting ($n = 5$); estimated error $\pm$ 5%.
[c]Not included in component band area calculations.

spectrum of adsorbed HSA (Fig. 3B), deconvolution reveals component bands at 1687, 1670 (shoulder), 1654, 1634, and 1615 cm^{-1}. Assuming solution state band assignments may be applied to surface-adsorbed protein (Wasacz et al., 1987; Lenk et al., 1989), tentative assignments of beta structure (1687 and 1634 cm^{-1}) and helix/aperiodic structure (1654 cm^{-1}) can be made (Parker, 1983; Susi and Byler, 1983; Byler and Susi, 1986; Jackson et al., 1989). In the spectrum of the adsorbed protein, additional broad bands not seen in the solution spectrum are observed at 1740 and 1708 cm^{-1} (data not shown). Bands in this region are not considered specific for protein backbone conformation, but rather are thought to arise from carbonyl groups of unionized carboxylic acid (Castillo et al., 1984). The appearance of bands in this region would be consistent with a change in the p*K*a's of the carboxylic acid groups of HSA brought about by a change in the structure of the protein upon adsorption (Haynes et al., 1994).

The contours of the observed infrared spectra are composed of several overlapping bands due to various protein segments existing in different secondary structures (Casal et al., 1988). The data in Table 1 list the quantitative contributions of each component band to the total Amide I contour of the original spectrum obtained by curve-fitting procedures (Casal et al., 1988). In the solution spectrum (Fig. 4A), a majority of the Amide I area is concentrated in the band at 1654 cm^{-1}, while most of the remainder is in the band at 1635 cm^{-1}. The band positions and relative area of the component bands would suggest that, in the solution state, a greater fraction of the HSA molecule exists in helix/aperiodic conformations and less in the beta-structure conformation. As noted previously, this is in qualitative agreement with the known secondary structure of human serum albumin (Carter and Ho, 1994). For the adsorbed HSA (Fig. 4B), a lesser fraction of the Amide I band is at 1654 cm^{-1}, while most of the remainder is at 1634 cm^{-1} (a beta-structure band). These results suggest that adsorption of HSA results in the appearance of beta structure at the interface. A gain in beta structure upon adsorption has also been observed by others for bovine serum albumin on copolyetherurethane urea (Lenk et al., 1989) and for lysozyme on hydrogels (Castillo et al., 1985).

The correspondence between the structure and the spectrum of a protein in an aqueous environment, both adsorbed and bulk state, is complicated by overlap in the infrared responses of helical and aperiodic conformations. The proportions of helix and aperiodic conformations in the solution and adsorbed state were estimated by

hydrogen–deuterium exchange (Hvidt and Nielsen, 1966; Olinger et al., 1986). In the presence of a large excess of D_2O, solvent accessible NH groups of the peptide bond will exchange hydrogen for deuterium to become ND groups. The Amide I band is composed primarily of hydrogen-bonded C = O stretching vibrations, the frequency of which is modified by bonding of the carbonyl oxygen to an adjacent deuterium. The interaction with deuterium shifts the characteristic frequency of aperiodic conformations to 1640–1648 cm^{-1}, effectively separating aperiodic absorbance bands from those of unexchanged helices. This technique is useful to separate helical from aperiodic spectral features only in those instances where one conformation is preferentially exchanged. The difference in rate of exchange of different conformations of proteins, including HSA, has been shown to be rather large; up to 24 h is necessary to fully exchange helical regions while aperiodic regions exchange almost instantly (Hvidt and Nielsen, 1966). Thus, with short times of exposure to D_2O (in the present experiments, less than 20 min), significant hydrogen–deuterium exchange of only aperiodic regions is expected.

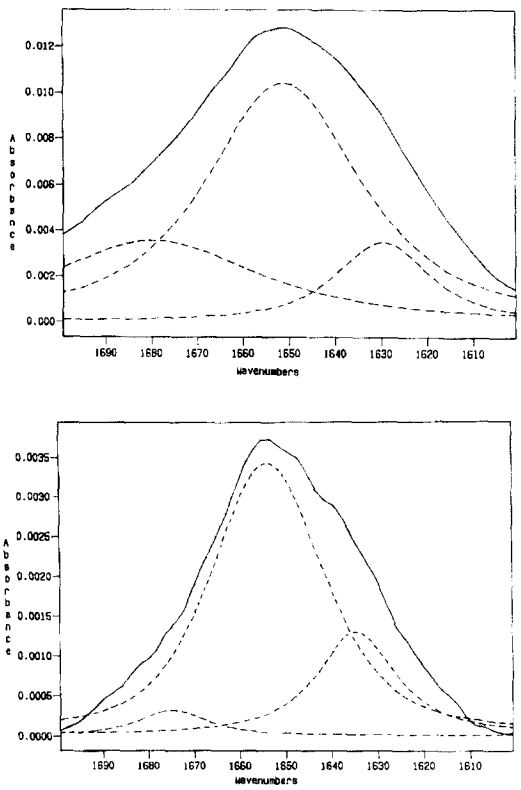

Fig. 4. Infrared spectrum of the Amide I region of human serum albumin in H_2O-based buffer. (A) Full line, spectrum of native protein in solution: dashed line, component bands of Amide I region of protein in solution. (B) Full line, spectrum of protein adsorbed to cast polysulfone film; dashed lines, component bands of spectrum of adsorbed protein.

The data in Table 2 show the Amide I component band frequencies and the quantitative contributions of each component band to the total Amide I contour for HSA in D_2O buffer. In the solution state after 20 min exposure to D_2O buffer, spectral deconvolution reveals component bands at 1672, 1653, 1645 and 1636 cm^{-1}. These bands are quite similar to those observed in H_2O-based buffer (Table 1), with the exception of the appearance of the new band at 1645 cm^{-1} comprising approximately 25% of the Amide I band. Since the 1645 cm^{-1} band appeared only after a brief exposure to D_2O, it can tentatively be assigned to aperiodic conformations. For the adsorbed protein exposed to D_2O buffer for 20 min, component bands at 1730, 1673, 1653, 1641 and 1638 cm^{-1} are observed. These bands are similar to those observed for adsorbed HSA in the presence of H_2O buffer (Table 1), with the exception of the appearance of the new band at 1641 cm^{-1}. Since the band at 1641 cm^{-1} also appeared only after brief exposure of the adsorbed protein to D_2O, it too can be tentatively assigned to aperiodic conformation. Band-fitting analysis indicates that for the adsorbed protein approximately 50% of the Amide I band area is due to aperiodic conformations. Compared to the band area assigned to aperiodic conformations in the deuterium-exchanged solution state, the increased area in the adsorbed state, along with the decreased area of bands assigned to helices, suggests that adsorption results in an appearance of aperiodic conformations at the expense of helical structures. In D_2O buffer, the changes in infrared bands assigned to beta structures are in agreement with those observed in H_2O buffer. After deuteration, both high frequency and low frequency component bands increase in area proportion upon adsorption, suggestive of an increase in beta structure in the adsorbed state.

Table 2
Characteristic Amide I component bands in the infrared spectrum of HSA in solution and adsorbed to PSf in the presence of D_2O buffer

	Observed wavenumber[a] (cm^{-1})	Proportion[b] (%)	Tentative assignment
Solution	1672	3	Beta structure
	1653	50	Helix
	1645	25	Aperiodic
	1636	22	Beta structure
Adsorbed	1730[c]		Carboxyl group
	1673	6	Beta structure
	1653	22	Helix
	1641	50	Aperiodic
	1638	26	Beta structure

[a]After deconvolution, see text for details.
[b]Average percent areas obtained by curve fitting ($n = 5$); estimated error $\pm 5\%$.
[c]Not included in component band area calculations.

The results of the present study indicate that, upon adsorption, the HSA molecule seems to gain aperiodic and beta conformations at the expense of helical structures. It should be noted that these data should not be interpreted to indicate that 50% of the HSA molecule in the adsorbed state adopts an aperiodic conformation. Unrecognized differences in infrared absorptivity of various components of secondary structure and the possible inaccessibility of D_2O to all portions of the molecule are complicating factors (Jackson et al., 1989; Surewicz and Mantsch, 1988).

While the observations of the present report do indicate a loss of helical structure upon adsorption to PSf, it is not yet possible to identify those specific regions of the molecule participating in the structural changes or those interacting directly with the surface. Consequently, the specific chemical groups acting between the protein and the surface, which are expected to influence membrane solute rejection and fouling, remain to be determined.

4. Conclusions

The structure of human serum albumin adsorbed onto a dense polysulfone film was studied in situ by Fourier-transform infrared spectroscopy. Amide I component band spectral positions and relative band areas show that, upon adsorption for 4 h from a flowing solution, HSA adopts a conformation containing less helical structure than the solution state. The protein is seen to gain aperiodic and beta conformations upon adsorption.

Acknowledgements

This work was supported by the NSF and the Commonwealth of Kentucky through the EPSCoR program (EHR-9108764). The author wishes to thank Prof. Kristine Knutson for helpful discussions.

References

Andrade, J., Principles of protein adsorption. In Andrade, J. (Ed.), *Surface and Interfacial Aspects of Biomedical Polymers*, Vol. 2, *Protein Adsorption*, Plenum Press, New York, 1985, pp. 1–80.

Baier, R.E. and Dutton, R.C., Initial events in interactions of blood with a foreign substance. *J. Biomed. Mater. Res.*, 2 (1969) 191–206.

Bird, R.B., Stewart, W.E. and Lightfoot, E.N., *Transport Phenomena*, Wiley, New York, 1960.

Bowen, W.H. and Gan, Q., Properties of microfiltration membranes: The effects of adsorption and shear on the recovery of an enzyme. *Biotechnol. Bioeng.*, 40 (1992) 491–497.

Brites, A.M. and dePinko, M.N., A new approach to the evaluation of the effects of protein adsorption onto a polysulfone membrane. *J. Membrane Sci.*, 78 (1993) 265–276.

Byler, D.M. and Susi, H., Examination of the secondary structure of proteins by deconvoluted Fourier-transfour infrared spectroscopy. *Biopolymers*, 25 (1986) 469–474.

Campbell, M., Walter, R.P., McLaughlin, R. and Knowles, C.J., Effect of temperature on protein conformation and activity during ultrafiltration. *J. Membrane Sci.*, 78 (1993) 35–43.

Carter, D.C. and Ho, J.X., Structure of serum albumin. *Adv. Protein Chem.*, 45 (1994) 153–204.

Casal, H.L., Kohler, U. and Mantsch, H.H., Structural and conformational changes of beta-lactoglobulin B: An infrared spectroscopic study of the effect of pH and temperature. *Biochim. Biophys. Acta*, 957 (1988) 11–20.

Castillo, E.J., Koenig, J.L. and Anderson, J.M., Characterization of protein adsorption on soft contact lens I. Conformational change of adsorbed human serum albumin. *Biomaterials*, 5 (1984) 319–25.

Castillo, E.J., Koenig, J.L., Anderson, J.M. and Lo, J., Protein adsorption on hydrogels. *Biomaterials*, 6 (1985) 338–345.

Chittur, K.K., Fink, D.J., Leininger, R.I. and Hutson, T.B., Fourier transform infrared spectroscopy attenuated total reflection studies of protein adsorption in flowing systems: Approaches for bulk correction and conformational analysis of adsorbed and bulk protein in mixtures. *J. Colloid Interface Sci.*, 111 (1986) 419–433.

Creighton, T.E., *Proteins. Structure and Molecular Properties*, Freeman, New York, 1984.

Devis, S., Terre, S., Bertheau, Y. and Boyaval, P., Factors affecting pertate lyase activity during membrane filtration. *Biotechnol. Tech.* 4 (1990) 127–132.

Fontyn, M., Bijsterbosch, B.H. and van't Riet, K., Chemical characterization of ultrafiltration membranes by spectroscopic techniques. *J. Membrane Sci.*, 36 (1987) 141–145.

Fontyn, M., van't Riet, K. and Bijsterbosch, B.H., Surface spectroscopic studies of pristine and fouled membranes. Part I. Method development and pristine membrane characterization. *Coll. Surf.*, 54 (1991) 331–347.

Hanemaaijer, J.H., Robbertsen, T., van den Boomgaard, Th. and Gunnink, J.W., Fouling of ultrafiltration membranes. The role of protein adsorption and salt precipitation. *J. Membrane Sci.*, 40 (1989) 199–217.

Haynes, C.A., Sliwinsky, E. and Norde, W., Structural and electrostatic properties of globular proteins at a polystyrene–water interface. *J. Colloid Interface Sci.*, 164 (1994) 394–409.

Hvidt, A. and Nielsen, S.O., Hydrogen exchange in proteins. *Adv. Protein Chem.*, 21 (1966) 287–386.

Ihlenfled, J. and Cooper, S.L., Transient in vivo protein adsorption onto polymeric biomaterials. *J. Biomed. Mater. Res.*, 13 (1979) 577–591.

Ishida, K.P. and Griffiths, P.P., Comparison of the Amide I/II intensity ratio of solution and solid-state proteins sampled by transmission, attenuated total reflectance, and diffuse reflectance spectroscopy. *Appl. Spectrosc.*, 47 (1993) 584–589.

Jackson, M., Haris, P.I. and Chapman, D., Conformational transitions in poly(L)lysine: Studies using Fourier-transform infrared spectroscopy. *Biochim. Biophys. Acta*, 998 (1989) 79–75.

Keurentjes, J.T.F., Harbrecht, J.G., Brinkman, D., Hanemaaijer, J.H., Cohen Stuart, M.A. and van't Riet, K., Hydrophobicity measurements of microfiltration and ultrafiltration membranes. *J. Membrane Sci.*, 47 (1989) 333–344.

Krimm, S. and Bandekar, J., Vibrational spectroscopy and conformation of peptides, polypeptides and proteins. *Adv. Protein Chem.*, 38 (1986) 183–364.

Lenk, T.J., Ratner, B.D., Gendreau, R.M. and Chittur, K.K., Infrared spectral changes of bovine serum albumin upon surface adsorption. *J. Biomed. Mater. Res.*, 23 (1989) 549–569.

Mantsch, H.H., Casal, H.L. and Jones, R.N. In Clark, R.J.H. and Hector, R.E. (Eds), *Spectroscopy of Biological Systems*, Wiley, New York, 1986.

Meirles, M., Aimar, P. and Sanchez, V., Albumin denaturation during ultrafiltration: Effects of operating conditions and consequences on membrane fouling. *Biotechnol. Bioeng.*, 38 (1991) 528–34.

Nilsson, J., Protein fouling of UF membranes: Causes and consequences. *J. Membrane Sci.*, 52 (1990) 121–142.

Nystrom, M., Fouling of modified and unmodified polysulfone ultrafiltration membranes by ovalbumin. *J. Membrane Sci.*, 44 (1989) 183–196.

Oldani, M. and Schock, G., Characterization of ultrafiltration membranes by infrared spectroscopy, ESCA, and contact angle measurements. *J. Membrane Sci.*, 43 (1989) 243–258.

Olinger, J.M., Hill, D.M., Jakobsen, R.J. and Brody, R.S., Fourier-transform infrared studies of ribonuclease in H_2O and $2H_2O$ solutions. *Biochim. Biophys. Acta*, 869 (1986) 89–98.

Palecek, S. and Zydney, A.L., Hydraulic permeability of protein deposits formed during microfiltration: Effect of solution pH and ionic strength. *J. Membrane Sci.*, 95 (1994) 71–81.

Parker, F., *Applications of Infrared, Raman and Resonance Raman Spectroscopy in Biochemistry*, Plenum Press, New York, 1983.

Pitt, A., The nonspecific protein binding of polymeric microporous membranes. *J. Parenteral Sci. Tech.*, 41 (1987) 110–113.

Pitt, W. and Cooper, S.L., Albumin adsorption to alkyl chain derivatized polyurethanes I. The effect of C-18 alkylation. *J. Biomed. Mater. Res.*, 22 (1988) 359–382.

Powell, J.R., Wasacz, F.M. and Jakobsen, R.J., An algorithm for the reproducible spectral subtraction of water from the FTIR spectra of proteins in dilute solutions and adsorbed monolayers. *Appl. Spectrosc.*, 40 (1986) 339–344.

Robertson, B.C. and Zydney, A.L., Protein adsorption in asymmetric ultrafiltration membranes with highly constricted pores. *J. Colloid Interface Sci.*, 134 (1990) 563–75.

Surewicz, W.K. and Mantsch, H.H., New insight into protein secondary structure from resolution-enhanced infrared spectra. *Biochim. Biophys. Acta*, 952 (1988) 115–130.

Susi, H. and Byler, D.M., Protein structure by Fourier-transform infrared spectroscopy second derivative spectra. *Biochem. Biophys. Res. Commun.* 115 (1983) 391–397.

van den Oetelaar, P.J.M., Mentinh, I.M. and Brinks, G.J., Loss of peptides and proteins upon sterile filtration due to adsorption to membrane filters. *Drug Dev. Ind. Pharm.*, 15 (1989) 97–106.

Wahlgren, M. and Arnebrant, T., Adsorption of beta-lactoglobulin onto silica, methylated silica and polysulfone. *J. Colloid Interface Sci.*, 136 (1990) 259–265.

Wasacz, F.M., Olinger, J.M. and Jakobsen, R.J., Fourier-transform infrared studies of proteins using non-aqueous solvents. Effects of methanol and ethylene glycol on albumin and immunoglobulin G. *Biochemistry*, 26 (1987) 1464–1470.